NYU SHANGHAI 上海 纽约大学

Pre-lab 6/1/15

No.
Date

Introduction to Transformation

- objectives:

i) Do the genetic transformation

ii) Determine the level of success

- procedures:

i) 2 micro test tubes, +pGLO -pGLO

ii) 250 μL of $CaCl_2$ in each

iii) Put em on ice

iv) pick 2-4 large, uniformly circular, smooth edged colonies of bacteria from starter plate using sterile loop. Dunk loop in a test tube and twirl it so no floating pieces. Place back on ice.

v) Look at pGLO solution w/ UV. New sterile loop, put it in stock sample and then dunk in +pGLO. Maybe pipette 10μL into +pGLO.

vi) Put tubes back on ice for 10 minutes

vii) Label four LB nutrient agar plates on the bottom: +pGLO: LB/AMP, LB/AMP/AG -pGLO: LB/AMP, LB.

viii) Heat shock: in foam rack put both tubes in 42°C water bath for 50 seconds. Immediately put on ice, keep for 2 minutes

ix) Put tubes on bench, add 250μL of LB into each. use new, sterile pipettes for both. Keep at room temp for 10 minutes

x) Flick closed tubes w/ finger, pipet 100 μL onto each correct plate.

xi) New loop for each, gently spread suspensions across pp. One at a time. Reduce contamination.

xii) Stack and tape together, place upside down in 37°C incubator.

- expected results:

i) +pGLO LB/amp/ara = could glow, LB/amp should be alive.

ii) +pGLO LB/amp/ara should be only one glowing

6/2/15

- Results:

i) None of the dishes glowed. Although colonies were apparent on 3/4 (not +pGLO LB/amp) dishes

↳ This is likely due to the several-month-old reagents we used no longer working effectively, then to human error

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6/10 Electrophoresis

No.
Date

- Objectives:
  1) Learn how to do GE
  ii) Determine size of DNA
- procedure (from Biotechnology)
  i) Gel onto tray, put in GE chamber. cover by ~2mm w/ TAE buffer
  ii) Note dyes and corresponding wells
  iii) Add 20 μL of dye to each corresponding chamber, gently.
  iv) After complete, put lid on and connect electrodes to terminals
  v) Electrophorese for 20 min at 100V
  vi) quickly record data bc gels diffuse quickly
- Procedure (from FB) for extraction
  i) column equilibriating: 500 μL BL to column CA2, spin 1 min, dispose liquid
  ii) weigh 1.5ml tube, put thin slice of gel in tube and weigh again
  iii) 3:1 vol:weight of PN to tube, incubate at 60°C for 5 min. Mix by inversion
  iv) cool solution, transfer to CA2, let stand 2 min. Spin 45 sec. Pour off liquid
  v) 600 μL PW, wait 2-5 min, spin 1 min, pour into beaker
  vi) Repeat v)
  vii) Spin 10 min @ 1300 RPM
  viii) put column in new EP tube, maybe air dry 10-15 min
  ix) Add 30-50 μL EB, let stand 2 min, spin 2 min, collect DNA
- procedure (from FB) for electrophoresis
  i) Add 0.4g agarose into big container
  ii) 100 mL TAE, swirl,

Date

iii) 2x0 for 3 min. Stop every 30s to swirl. Boil to dissolve
iv) cool to 55-60°C. add 5μL DuRed & swirl to mix
v) Prepare casting apparatus, pour molten agarose into tray, let solidify 15-20 min
vi) Remove comb
vii) Label microfuge tube for each sample
viii) Add 1μL of 6X dye, 5μL DNA, Pipet up and down to mix
ix) Run gel under water to saturate wells, put gel in chamber, pour buffer to 5mm cov
x) Load 5μL of 1kb ruler into lane 1
xi) Load 6μL mixture w/ loading dye into wells
xii) Connect to power supply, turn on, make sure wells next to −, 1hr @ 100V
xiii) Make sure dye doesn't run off
xiv) Look @ gel and record results

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6/15/15

Transformation

No.
Date

- objective:

1) transform promoters/RBS/chromoproteins/Backbone

- procedure

I) Thaw competent cells on ice

II) 50 µL thawed competent cells into chilled 2mL tube. ~~Gently pipet~~ & control

III) 1-2 µL of resuspended DNA to 2mL tube, pipet ↓↑ 1µL Rfp to control

IV) Incubate on ice for 30 mins

V) Heat shock @ 42°C for 1 min

VI) Incubate on ice for 5 mins

VII) 200 µL SOC media

VIII) Incubate @ 37°C while shaking for 2hr

IX) Label dishes, plate 20µL (rest) → control and 200 µL of trans. and spread

X) Incubate @ 37°C for 12-18 hrs, agar side up.

6/18/15

NYU SHANGHAI 纽约大学 上海

No.
Date

Restriction Digest

- Objective:

1) Successfully perform a restriction digest

- Procedure

I) Everything stays on ice

II) Thaw NEB 2, BSA in Room temp H_2O. Mix by shake

III) 280 ng DNH to tubes, Add dH_2O for $V_{tot} = 16\mu L$

IV) 2.5 μL NEB 2 to each

V) 0.5 μL BSA to each

VI) tube A: 0.5μL ~~E~~ X, 0.5μL S Tube B: 0.5μL X, 0.5μL P

VII) PSB1A3/PSB1A2 tube: 0.5μL: E, P, D

VIII) should have ~20μL in each tube. Pipet slow to mix, spin briefly.

IX) Incubate 37°C for 30 min, 80°C for 20 min

X) ~2μL of digest for ligations

Ligations

1) 2μL of digest to plasmid backbone, part C (25ng)

II) equimolar amt. A: E-HF, S digest (4.3 μL) B: X, P digest (4.3 μL)

III) 1 μL T4 ligase buffer

IV) 0.5μL T4 ligase

V) Water to $V_{tot} = 10\mu L$

VI) Ligate 16°C 30 min, heat kill 80°C 20 min

VII) transform w/ 1-2 μL of product

6/18

NYU SHANGHAI 纽约大学 Restriction Digest w/ NEB 2/2.1 No. Date

iGEM protocol:

2.5 uL NEB 2, 0.5 uL BSA

NEB2: 50mM NaCl, 10mM Tris-HCl, 10mM $MgCl_2$, 1mM DTT

NEB 2.1: 50mM NaCl, 10mM Tris-HCl, 10mM $MgCl_2$, 100ug/mL BSA

Need 0.5 uL at 100 ug/mL = [illegible]

2.5uL NEB2.1 x 0.1 mg/mL = 0.25 ug BSA

BSA: [illegible]

→ Using ½ amt. of BSA that is called for

DTT: Need [illegible] 50mM

NEB 2:

NaCl = 58.44 g/mol 500 mM = ½ M

Tris-HCl = 157.56 g/mol 100 mM = 1/10 M

$MgCl_2$ = 95.211 g/mol 100 mM = 1/10 M

DTT = 154.253 g/mol 10 mM = 1/100 M

$154.253 \frac{g}{mol} \times 2.5 uL \times .01 \frac{mol}{L}$

$2.5 \times 10^{-6} L \times .01 M \frac{mol}{L} \times 154.253 \frac{g}{mol} =$

$= .003856 mg = 3.856 ug$

1M = 1.54 g / 10 mL H_2O

0.01 M = 1.54g / 1L H_2O = 0.154g / 100 mL = 0.0154g/10mL

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6/19 RE D

No. 2019 3P
Date 6.5 3P

1) 2.5 μL NEB 2.1 to ~~each~~ 250 ng DNA + H_2O @ 16 μL

II) 2.5 μL DTT to ~~each~~ 250 ng DNA + H_2O + NEB 2.1 @ 18.5 μL

III) 0.5 μL PstI, 0.5 μL ~~EcoRI~~ X 6.7 to 21.0 μL

IV) have 22 μL, mix well & spin briefly

V) 37°C for 30 min, 80°C for 20 min

VI) Run gel

250 ng DNA @ 57 ng/μL = 4.385 μL DNA

& 9.615 dH_2O

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Ligation Protocol (iGEM)

6/23/15

No. 10:00
Date

- Objectives:

1) Ligate fragments separated by restriction digest

- Procedure:

I) 2 μL (25 ng) of digested plasmid backbone
II) Equimolar EcoRI-HF | SpeI fragment (<3 μL).
III) Equimolar XbaI | PstI fragment (<3 μL)
IV) 1 μL T4 DNA ligase buffer (Not quick ligase)
V) 0.5 μL T4 DNA Ligase
VI) dH_2O to V = 10 μL
VII) 16°C for 30 min, 80°C for 20 min
VIII) Transform w/ 1-2 μL of product

- Note:

1) DpnI is used previously to prevent red colonies from forming w/ JO4450
II) 25 ng/μL DNA = .025 $\frac{g}{L}$

6/24/15

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Restriction Digest of green chromoprotein

No.
Date

- Need: 155.38 ng of K592011 (Green chromoprotein)

@ 82.3 $\frac{ng}{\mu L}$ = 1.888 μL of DNA

2 μL EcoRI buffer

0.5 μL EcoRI enzyme

0.75 → 1.0 μL SpeI enzyme

20 μL dH_2O

= 25.39 μL V_{tot}

Trial 1

Fragment 1: E-GCP-S = 725 BP

Fragment 2: S-PSB1C3-E = 2047 BP

↳ Trial 2: Same conditions/amts. etc except using NEB 2.1 (2μL) instead of NEB EcoRI Buffer (2μL)

- Then incubate for ~~1 hour~~ 75 minutes @ 37°C

↳ While incubating, preparing gel* in order to run samples

↳ 1 kb ruler (6μL) and 5μL samples + dye 100 v for 1 hr, should have bands at 2kb and ~700BP

- Results:

i) Ran gel w/ 1% agarose for 30 min (time constraint) saw bands at 2kb and 700 for both samples although 2.1 was clearer than NEB EcoRI Buffer.

ii) Everything turned out as expected

6/29 Monday

SHANGHAI

3A assembly

Materials: 0.6477 μL K081005

0.4387 μL K1033916, 0.9117 μL E0040, E/X/S/P enzymes,

buffers: EcoRI, 2.1, 3.1, T4 Ligase. Need T4 ligase enzyme

- Plasmid 1: K081005: promoter + RBS @ 153.9 ng/μL

i) 2 trials; use ~~2.5 μL~~ EcoRI Buffer / ~~2 μL~~ NEB 2.1

ii) 0.6477 μL DNA + ~~8.8523~~ 7.7523 μL dH_2O

iii) ~~1.5~~ 1.0 μL Buffer, then 0.20 μL EcoRI + 0.40 μL SpeI

- Plasmid 2: K1033916: YG chromoprotein @ 227.2 ng/μL

i) 1 trial w/ NEB 3.1 buffer

ii) 0.4387 μL DNA + ~~8.5613~~ 8.5613 μL dH_2O

iii) Add ~~over 1.5~~ over 1.0 μL buffer, then 0.2 μL XbaI + PstI

- Linearized Backbone: E0400, GFP in AMP @ 106.6 ng/μL

i) 3 trials, use EcoRI Buffer / 2.1 / 3.1

ii) 0.9117 μL DNA + 7.6883 μL dH_2O

iii) Add 1.0 μL buffer, then 0.2 μL EcoRI / PstI

- Incubate @ 37°C for 1 hr, then kill @ 80°C for 20 min

- Ligate: 1 μL each ~~fragment~~, 0.~~5~~4 μL ligase buffer, 0.25 μL ligase, ~~0.25~~ 0.4 μL dH_2O. Ligate @ 16°C for 30 min then 80°C for 20 min

transform w/ 1 μL product

$V_{tot} = 4\,\mu L$

Tuesday 6/30

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Linearizing Plasmids

No. pSB1C3
Date pSB1A2
EcoRI, PstI,

- Plan is to make 3μg of DNA of each plasmid.
- Plasmids: CFP in pSB1C3, E0020, 4N, 22.4132μL tot @ 180.6 ng/μL
  gfp in pSB1A2, E0040, 13L, 2 conc.: 18.9718μL @ 106.6 ng/μL = TUBEA
  25.1806μL @ 80.2 ng/μL = TUBEA2
- 4 tubes in total, 2 for each antibiotic. V_{tot} for each tube = 50μL
  pSB1C3 tubes have 11.2066μL of DNA, pSB1A2 have 18.9718 and 25.18
- Enzymes: EcoRI, PstI, 40.448 units for antibiotic = 20.224 unit/tube
  ≈ 1μL of each enzyme per tube
- Buffers: NEB 2.1, 5μL/tube.
- Water: pSB1C3: 31.7934μL/tube. TUBEA1: 24.056μL, TUBEA2: 17.819μL
  so V_{tot} of each tube is 50μL
- Incubate: 37°C for 1 hour, 80°C for 20 min.

Wednesday 7/1/15

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Gel extraction

No. Linearized pSB1C3/pSB4A2
Date

- use lower (0.7-0.8%) gel at lower V for longer time for best results
- Extraction: use glass plate if possible, and minimize time spent under UV
- Weigh empty tube, zero it, then weigh gel slice. Minimize excess gel.
- Now we purify the gel

- Purification: Tiangen protocol

I) 500 µL Buffer BL to S.C. CA2 (put in collection tube). Spin 1 min @ 12K, discard FT

II) Add equivolume PN buffer to gel [0.1g gel = 100µL], then incubate at 50°C and invert tube repeatedly till gel dissolves.

III) Sample at room temp, transfer to S.C. CA2. Let stand 2 min, Spin 45s @ 12K

Note: max V is 800 µL. If more, just load again.

IV) Wash S.C. CA2 w/ 600 µL BPW (w/ ethanol), spin 45s at 12K, discard FT.

V) Repeat step IV)

VI) Put scCA2 back in collection tube, spin 2 min @ 12K to remove residue. discard FT, let dry w/ cap open for several minutes

VII) Transfer scCA2 to 1.5mL tube. Add appropriate * volume BEB, incubate at room temp 2 min. Spin 2 min @ 12K,

1.484 - 0.915 = 0.569 g

Sample 2: 90 µL EB ✓

Sample 1: 80 µL EB ✓

1.2353 - 0.917 / 9

.31829

Gel extraction & 3A assembly

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Friday, July 3

No.
Date

- Linearized pSB1A2 extraction w/ MinElute protocol
- 3A assembly w/ linear pSB1A2, YG chromo, pRBS
- For extraction, used ~~APP~~ 54 mL of 50 mL (cut) + 10 mL dye in x1 cut out 3x 18 mL bands, should be ~ $\frac{45}{50} \times \frac{300}{2}$

0.6925 mL amp BB pSB1A2
1.27766 mL pRBS K880005
1.66126 mL YGFP K1033916
1 mL T4 Buffer
0.5 mL Ligase
4.86857 mL dH_2O

30 min @ 16 C, then 20 min @ 80 C

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Tuesday 7/7

Digest & Ligation (3A)

No.
Date

* Re-do ~~Negations~~ Digests of pRBS & YGCP in order to get better concentration for 3A. Then increase concentrations of everything in 3A

Digests: [Reaction A] | [Reaction B]

[Reaction A]	[Reaction B]
2.57 mL pRBS @ 136.4 ng/mL	1.54 mL YGCP @ 227.2 ng/mL
0.1 mL EcoRI & SpeI	0.1 mL XbaI & PstI
1.25 mL buffer 2.1	1.25 mL buffer 3.1
4.94 mL dH_2O	5.96 mL dH_2O
$V_{tot} \approx$ 10 mL w/ 350 ng pRBS	$V_{tot} \approx$ 10 mL w/ 350 ng YGCP

Then incubate for ~1 Hr @ 37°C, 1% 80°C 20 min

3A:

1.385 mL pSB1A2 linear for 50 ng

2.839 mL pRBS K880005 for 102.213 ng

3.692 mL YGCP K1033916 for 132.9 ng

0.5 mL DNA T4 ligase

1.0 mL DNA T4 ligase buffer

2.089 mL dH_2O

Incubate @ room temp for 1 hr

Incubate overnight @ 4°C

Freeze @ -20°C till transformation

Monday 7/13

NYU 上海 SHANGHAI 纽约大学 Transformation of 3A assembled pRBS/YbCP/pSB1A2

No.
Date

◆ Objective: Transform my ligated plasmid into DH5α cells

- 50 μL DH5α, 5 μL ligation, 200 μL SOC media

• Procedure:

I) After thawing DH5α, add 50 μL of it to 2 chilled 2mL tube

II) 5 μL of DNA (ligation) in each. one is control

III) Incubate closed tubes for 30 min on ice

IV) Heat shock 42°C for 60 sec, then 5 min on ice

V) Add 200 μL non-contaminated SOC media to both tubes

VI) Incubate at 37°C for 2 hours

VII) Label LB/Agar and LB/Amp/Agar plates. ~~200~~ 185 μL to plate with antibiotics, ~~150~~ 65 μL to agar plates. Spread.

VIII) Incubate 37°C for ~18 hours, Agar side up.

7/14

- After 17 hours of incubation, both control and antibiotic plates had lots of growth.

Monday 8/3/15 digest + Ligation
Blue chromoprotein

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No.
Date

Digestion: BBa_K592009: Blue chromoprotein (amilCP)
concentration of miniprep: 121.3 ng/μL
Want digest concentration of 35ng/μL, V_{tot} = 10 μL
350 ng / 121.3 ng/μL = 2.885 μL DNA
4 0.1 μL XbaI + PstI
4 1 μL 3.1 Buffer = 10μL V_{tot}
4 6.0146 μL dH_2O
mix, incubate 1hr @ 37°C, then heat kill 20 min @ 80°C

After digestion, ligate w/ PSB1A2 (linear) + pRBS (digested) from 7/7.
1.38 μL backbone, 2.86 μL pRBS, 3.78 μL Blue CP, 1μL ligase Buffer
0.5 μL ligase, 0.48 μL dH_2O V_{tot} = 10 μL
Incubate @ room temp for 1 hour, then overnight @ 4°C.

8/4

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No.
Date

Digest: 2.5 mL YGCP @ 227.2 ng/mL w/ EcoRI + PstI

concentration = 56.8 ng/mL of DNA = 42.554 ng/mL of backbone

= 425.54 ng backbone total

incubate 1hr @ 37°C, then 60°C for 20 min

- Gel: Made 0.8% Agarose gel, ran for 45 minutes at 75V.

- Extraction: cut out 1 slice of backbone; tube weighed 0.9117g, with gel = 0.9884 g.

weight of gel = 76.7 mg. Now purification, w/ Qiagen protocol

4) 76.7mg gel = 230.1 mL buffer QG. Then incubate 10 min @ 50°C

- 76.7 mL isopropanol after gel dissolves
- put in provided tube and then spin 1 min @ 13k rpm
- ~~Discard, let sit 5 min~~ discard → 750 mL ethanol buffer, spin, let sit 5 min, repeat w/ ethanol. ✡ After initial spin & discard, spun again for 20 seconds.

✡ Heated elution buffer in 80°C bath before elution

- Clean 1.5mL tube, 10 mL heated EB buffer, let stand 1 min and then spin 1 min.
- take flow through, put back in and spin again

- Nanodropped → 23.1 ng/mL $\frac{260}{280}$ = ✓

- Transformation: used protocol from 7/13 w/ 3.5mL.

- Inoculation: 12 μL SOC / tube, 12 mL antibiotic

transformed PRBS & Blue CP + Amp

inoculated PRBS + YGCP + Amp

8/5/15 Working w/ overdiluted IDT stevene

- Made a 0.8% gel in order to run the overdiluted blue chromoprotein that IDT sequenced for us. Plan is to extract it and get a ~100's yield to increase the concentration by 3X.
- Gel: going to run 30 mL @ 10ng/mL of blue chromoprotein for ~45 min at 75 V, then extract digest in my chloramphenicol backbone
  1.45g - .9115 = 0.5335g = 536 mg = 72 pericentrons
  ↳ 1200 µL QG → 10 min @ 50°C
  ↳ 400 µL isopropanol, then pipette column, then spin
  ↳ 500 µL QG, then spin
  ↳ 750 µL PE, then spin, then let sit
  ↳ Spin 1 min, then 1.5 mL tube. put 70°C EB in at 10 mL, then let stand. spin, put back, stand, spin
- Wasn't so great.
- Digestion and ligation: IDT blue ci w/ BB and YFP amp w/ BOOM and chloramphenicol BB, after Rachel minipreps the pRBS & YGCP & Amp.
  ↳ IDT BCP: 10 µL @ 10ng/µL, ran no gel 1.0 mL 2.1 & 0.15 mL EcoP. 15 minutes @ 37°C, then 20 @ 40°C. Ligate w/ 8.85 µL digestion, so 7.3 µL BCP + 1.0 µL T4 buffer & 0.5 µL ligase and 1.2 µL chlor. backbone. 1 hr @ room temp then overnight @ 4°C
  ↳ YGCP → depends on Rachel's miniprep results...

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8/5 cont.

- Terminator Ball conc. 82.5 $\frac{ng}{\mu L}$ ... 5 mL + 1.0 mL 3.1 + 0.15 mL X + P + 3.7 mL dH_2O = $\frac{412.5\,ng}{10\,mL}$ = 41.25 $\frac{ng}{\mu L}$
- Rachel's miniprep = 350 $\frac{ng}{\mu L}$; 1.43 μL for digest = $\frac{500\,ng}{10\,mL}$
7.3 mL H_2O + 1.0 mL 2.1 + 0.15 mL E+S = Reaction
- Ligation of Rachel's miniprep digested + terminator
  - 2.486 mL chromoprotein
  - 2.164 mL backbone
  - 2.278 mL terminator
  - 1.0 mL T4 buffer
  - 0.5 mL Ligase
  - 1.156 mL H_2O
- Ligations: Room temp 1 hr, then overnight @ 4°C
- inoculations: ligated blue chromoprotein
  - 12 mL SOC, 12 μL ampicillin, pipette tip of colony

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8/6 Digest, extract, transform

No.
Date

- Yesterday's innoculations didn't turn out so well... the shaker thing turned off after we left so the samples sat at room temp for ~18 hours
- Today: digests to extract backbone; 4 tubes, 2 samples
  ① 7.0 mL lime chromoprotein in pSB1C3 @ ~100 ng/mL, 1.3 mL E+P+2.1, 2.7 H_2O
  ② 20 mL PRBS+YGCP temp @~350 ng/mL, 1.3 mL E+P+2.1, 6.7 mL H_2O
  1 hr @ 37°C then 20 min @ 80°C.
- Make 0.8% gel, gonna run 8 lanes w/ 5mL sample and 1mL 6X dye per lane, 4 for each backbone. ~270 ng BB/lane
- 45 ~ 1 hr @ 75 V

R - CCCC -- AAA ----

C_1 = 174.1 ng/μL C_2 = 148.2 C_3 = 196.5 C_4 = 108.8

5 22.3 ng/μL Q6 444.6 589.5 326.4

- only #4 is usable.

Also transformed 3 mL of IDT plve and my own YFCP in 4 tubes, should have 8 plates total.

- Started MiniPrep; centrifuged and then freeze

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8/7

No.
Date

- Yesterday's transformation worked, as in there are colonies on both plates, except there's no color. I added arabinose to the IDT one, hopefully there will be color this afternoon.
- This morning I am repeating yesterday's digests in order to extract backbones again. Hopefully less distractions today.
- Gel for running the digestions is 0.8%.
- After extraction, I've determined that there is a problem with my Ampicillin sample.

Tossing all four; chloramphenicol:

$C_1 = \frac{1.0961}{1.2916}$: 195.5 mg C_2: 124.2 mg C_3: 130.0 mg C_4: 127.9 mg

586.5 mL 372.6 mL 390.0 mL 383.7 mL

↳ C_1 was good, C_2 was okay, C_3 was a great and C_4 was bad.

IR-LVL1-CAT1-12

Monday 8/10

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No.
Date

- More extraction today. This morning, digestions ~~rotate~~ and gel making.
- Digests: 2 tubes, 1 with 14 mL lime chromoprotein in pSB1C3 and the other with 12 mL pSB1A2 backbone concentrations ~ 105 ng/mL. V_{tot} = 20 mL; 2.6 mL 2.1 & EtP, rest filled w/ water. 1hr @ 37°C then heat kill @ 80.
- Gel: 0.8 % Agarose; gonna run 2 wells for each antibiotic resistance w/ 10 mL digest + 2 mL 6X dye.
- Extractions:

— CC--AA-R

$A_1 = \frac{1.2535}{1.1020} = 151.5$ mg = 454.5 mL

$A_2 = \frac{1.9342}{1.1058} = 225.4$ mg = 676.2 mL

$C_1 = \frac{1.2475}{1.1021} = 145.4$ mg = 436.2 mL

4 Extraction results were pretty good.

Tuesday 8/18 Transformation Tuesday

SHANGHAI JIAO TONG UNIVERSITY digest, ligation, transformation No. Date

Ligate pRBS + Amil Blue CP in Amp w/ a terminator and chloramphenicol backbone after digests. Then transform on agar plate.

Digests: 2 tubes one w/ ~100 ng/mL pRBS + blue CP in amp, 5 mL + 1.3 mL EcoRI-HF, + 3.7 mL dH_2O. other w/ ~85 ng/mL BOOM terminator in amp, 5 mL of it + 1.3 mL XbaI + 2.1, + 3.7 mL dH_2O. 37°C for 55 min then ice for 1 hr, lunch, then 20 min @ 80°C

Ligate w/ 2 mL of 23.1 ng/mL chloramphenicol BB, #2 mL of each digest, 1 mL T4 buffer, 0.5 mL T4 ligase 4.5 mL dH_2O.

Transform w/ 200 mL LB, not SOC. 25 min on ice, then 1 hr 50 min on ice after heat shocked 4 plates, 125 mL each, 2 control (LB) 2 LB+Chl

Wednesday 8/19

inoculation
Resuspension
Transformation

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No.
Date

- inoculating yesterday's amilCP blue full that didn't show color, in order to miniprep and have it resequenced
- Resuspending and transforming RFP in a low-med copy plasmid.
  10 mL ultrapure water in plate kit 4, well 4D, J04450
- transforming 2.5 mL ~~dbr~~ w/ 50 mL dH5α
  2 Plates, LB+ chlor. Should glow red in 18 hrs
- inoculation w/ 2 tubes, miniprep tomorrow
  12 mL LB/tube + Pipette tip + ~~24~~ 24 mL of 12.5 $\frac{mg}{mL}$ chloramp
- Transformation: 1hr 45 mins in shaker, 125 mL/plate